Structural and Functional Diversity of β-Adrenergic Receptors[a]

A. D. STROSBERG

Institut Cochin de Génétique Moléculaire
Laboratoire d'Immuno-Pharmacologie Moléculaire
CNRS UPR 0415
22, rue Méchain
75014 Paris, France

and
Université de Paris VII
Paris, France

Recent progress in molecular cloning techniques has allowed the identification of the genes or cDNAs corresponding to proteins homologous to but different from previously known receptors. These "new" sequences have since been shown to encode subtypes whose existence had been suspected on the basis of pharmacologic data. A good example of such a new subtype is the β_3-adrenergic receptor (β_3AR). Its gene was first cloned by homology using β_2- and β_1-specific DNA oligonucleotide probes.[1] Corresponding β_3 species homologues were later cloned from mouse[2] and rat.[3,4] After transfection of the β_3AR gene in Chinese hamster ovary (CHO) cells, which are devoid of such receptors, it was shown that the βAR subtype displays most of the ligand-binding and adenylyl cyclase-stimulating properties of previously described "atypical" receptors in various rodent species (reviewed in refs. 5 and 6).

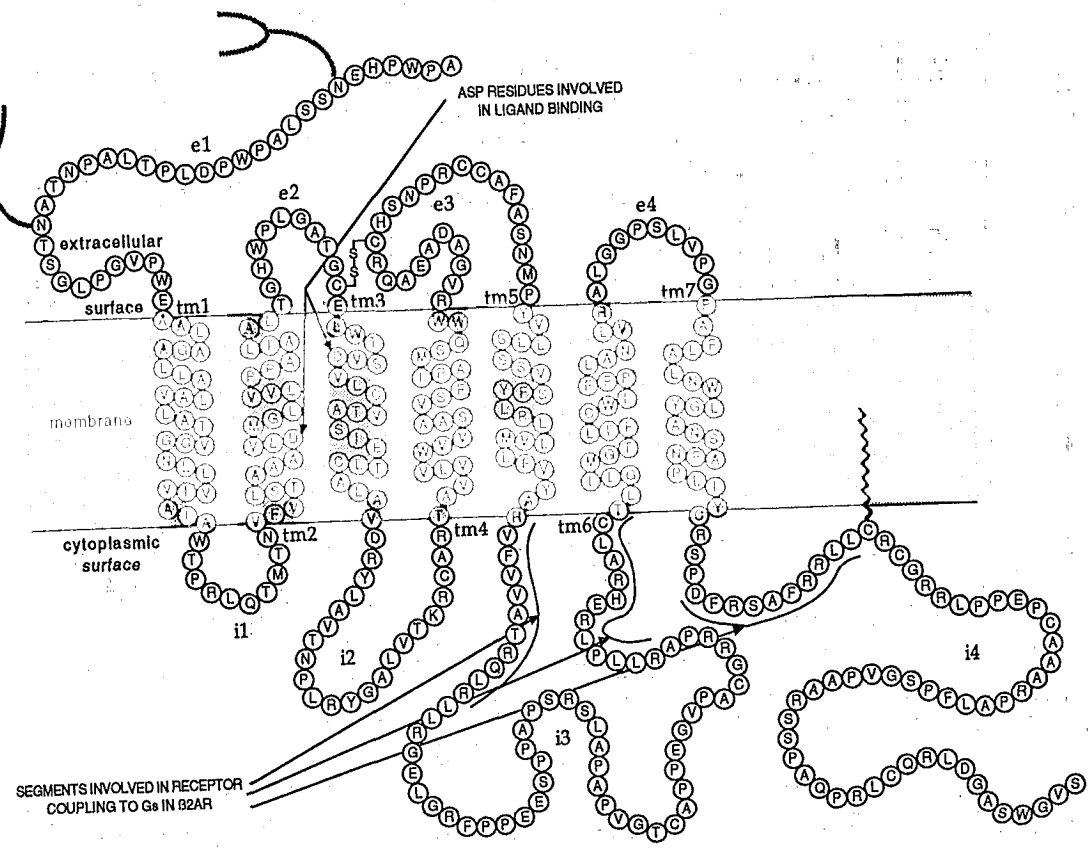
The β_3AR is quite distinct from the β_1- and β_2AR and thus presents an interesting case of diversity in the small family of βARs.[7] In this paper we discuss the various features that distinguish the three βAR subtypes.

MOLECULAR DIFFERENCES

All three βARs belong to the R_7G superfamily of receptors coupled to GTP binding proteins.[8] They are thus composed of a single polypeptide chain with seven putative transmembrane domains, an extracellular glycosylated N-terminal and an intracellular C-terminal region (FIG. 1). As in all other R_7G proteins, the βAR subtypes vary extensively both in terms of length and sequences in the N- and C-terminal as well as in the third intracellular (i3) regions (FIG. 2). Although the seven hydrophobic regions are quite conserved, a number of differences may explain the striking pharmacologic and regulatory variations.

The degree of amino acid sequence identity between man, mouse, and rat β_3AR (FIG. 2) is much higher (80–90%) than that existing between different βAR subtypes

[a]This work was supported mainly by the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the University of Paris VII, the Ministry for Research and Technology, and the Bristol-Myers-Squibb Company. We are also grateful for support from the Ligue Nationale contre le Cancer, the Fondation pour la Recherche Médicale Française, and, last but not least, the Association pour la Recherche contre le Cancer.



(40–50%) and is of the same order as that observed across species for a given receptor subtype. Several residues located in the functional domains of the receptor are specifically shared by the human, murine, and rat β_3AR, but are not found in the β_1- and β_2AR sequences. From a genetic point of view, the human, mouse, and rat β_3AR genes all display a similar genomic organization with an intron interrupting the 3′ end of the coding block,[9–12] whereas the other βAR subtypes are intronless. Moreover, the human and mouse genes have been assigned to a chromosomal linkage group which is conserved between the two species.[2] Together, these structural data strongly suggest that the human, mouse, and rat genes do not encode distinct βAR subtypes,[4] but are species homologues of the β_3AR gene.

A number of residues found in human β_1- and β_2AR sequences are substituted by residues that appear to be specific for β_3. Most of these residues are found in all β_3-receptors sequenced so far. Some of the changes appear to be selective for the human β_3. Last, but not least, the Ser/Thr phosphorylation target sites documented for β_1- and β_2ARs are absent from all β_3. The rodent β_3ARs appear to share with the human β_3 quite a number of structural features that distinguish this subtype from β_1 and β_2. There are, in addition to these β_3-specific substitutions, a number of rodent specific changes, as is the case, for example, for the Val Ala Leu deletion in the tm1 domain.

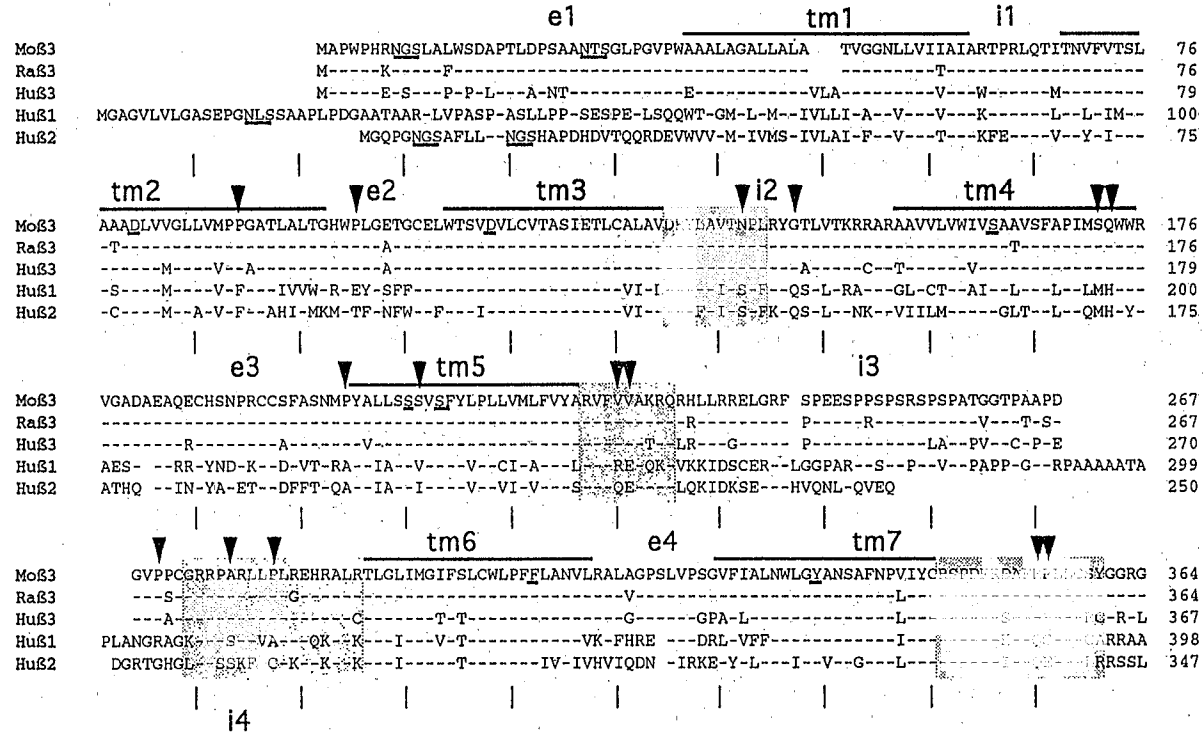
PHARMACOLOGIC DIFFERENCES

Five major features distinguish the human β_3AR from those of β_1 and β_2: (1) atypically low affinity for conventional β-antagonists including reference radioligands; (2) atypically low stereoselectivity index for agonist and antagonist enantiomers; (3) atypically low potencies of reference agonists; (4) high potency of a novel class of compounds initially described as potent activators of lipolysis and thermogenesis in white and brown adipose tissues of rodents; and (5) partial agonistic activities of several β_1/β_2-antagonists, reflecting intrinsic sympathomimetic activities in tissues. Such general properties are reminiscent of those ascribed to the atypical βAR initially proposed to exist in adipose tissues and later in the digestive tract of rodents. These tissues are also the only ones where β_3 mRNA expression was unambiguously demonstrated both in human[13] and rodents.[2,3,12]

The rodent β_3 again appears to be somewhat different from the human subtype: in CMO β_3 cells, propranolol is an antagonist for mouse β_3, as it is for β_1 and β_2, in contrast to what is seen in the human β_3 where it behaves as a weak agonist. A number of other compounds display either stronger or weaker effects in the β_3 human or rodent without any obvious trend: BRL 37344, for example, approximately 30 times more potent in the rodent, whereas carazolol is about 8 times more potent in the human β_3.

←

FIGURE 1. Primary structure of the human β_3AR. The sequences are represented in the one-letter code for amino acids. The single polypeptide chain is arranged according to the model for rhodopsin. The disulfide bond essential for activity Cys^{111} and Cys^{109} is represented by -S-S-. The two N-glycosylation sites in the amino-terminal portion of the protein are indicated by ⅄. The palmitoylated Cys^{360} residue in the N-terminus of the i4 loop is indicated by the symbol ≀ .



DIFFERENT CONTROL MECHANISMS REGULATE β_1-, β_2- AND β_3AR EXPRESSION

A variety of control mechanisms regulate the function and expression of the βAR subtypes. We summarize in TABLE 1 a few of the factors involved in βAR regulation, and show the differences between β_1, β_2, and β_3.

Agonist-induced Desensitization

One of the most specific mechanisms is desensitization, a complex physiologic process, which prevents the hormonal overload of most G-protein–coupled receptors by impairing the signal-transmission pathway at receptor and/or postreceptor levels. An important clinical consequence of desensitization is the relative or complete resistance to pharmacologic agonists given for an extended period of time.

Several molecular mechanisms involved in receptor desensitization have been characterized for the β_2AR.[14] After a few minutes of receptor activation by an agonist, phosphorylation of β_2AR by protein kinase A (PKA) and βAR kinase (βARK) results in the rapid uncoupling of the receptor from the transducing pathway.[15,16] Phosphorylation by PKA is a negative-feedback of receptor activation, mediated by the raise of intracellular cAMP, which affects all β_2AR, whereas βARK phosphorylates only those receptors that are occupied by the agonist.[17] When receptor activation is sustained for longer periods of time (hours), protein degradation of preexisting receptors and destabilization of the receptor mRNA contribute to the reduction of the total number of β_2AR (i.e., receptor down-regulation).[18]

The three βAR subtypes are not equally sensitive to desensitization. The β_1AR, which has fewer potential phosphorylation sites and lacks the two tyrosine residues implicated in the down-regulation of the β_2AR,[19] is less prone than the β_2AR to both short-[20] and long-term[21] desensitization. The third βAR subtype is almost completely resistant to short-term desensitization,[12,22] primarily because this receptor does not undergo PKA- or βARK-induced phosphorylation—most likely because of the absence of the target sequences identified on the β_2AR.[8]

Other Mechanisms for βAR Modulation

A number of other compounds and factors have been shown to affect βAR expression and function (TABLE 1). These include glucocorticoids, butyrate, insulin adipocyte differentiation, and cold. The three βARs are diversely affected by these different conditions, again demonstrating that each subtype is regulated independently and may thus play a distinct physiologic role.

FIGURE 2. Amino acid sequence comparison of the mouse (Mo), rat (Ra), and human (Hu) β1-, β2-, and βARs. The seven transmembrane segments (tm1–tm7) are boxed and alternate with extracellular (e1–e4) and intracellular (i1–i4) domains. Gaps (*horizontal dashes*) have been introduced to maximize sequence alignment. Black triangles indicate residues conserved in all nine proteins, and dots represent classical substitution according to Daihoff PAM 250 matrix.

CONCLUSION

The comparison of the three βAR subtypes reveals both striking structural similarities, especially in the ligand binding and G-protein interacting sites, and important differences. These concern mainly regulatory control of receptor expression and function. It is likely that other families of receptor subtypes will display similar features by which binding of the same natural ligands may result in widely different functional effects, well controlled by developmentally or hormonally regulated mechanisms.

SUMMARY

The molecular and functional properties of the three βAR subtypes appear to be quite diversified even though all three bind the same natural catecholamines, adrenaline and noradrenaline, couple apparently to the same G_s transducer protein, and stimulate the same adenylyl cyclase effector. Binding characteristics for a variety of synthetic ligands thus encompass a wide range of K_d values, and a number of β_1/β_2 antagonists turn out to be potent agonists towards the β_3 subtype.

TABLE 1. Differential Up- and Down-Regulation of the Three β-Subtypes

Factors	β_1	β_2	β_3	References
Adipose differentiation	↓	↓	↑	Fève *et al.*[23,24]
cAMP	↓	↓	↑	Thomas *et al.*[25]
Dexamethasone	↓	↑	↓	Fève *et al.*[23,26]
Butyrate	↑	↑	↓	Krief *et al.*[27]
Insulin	→	→	↓	Fève *et al.*[28]

Regulatory mechanisms also appear to vary considerably from one β-receptor to another, with dexamethasone, for example, up-regulating the β_2 and down-regulating the β_1 and β_3 subtypes. Most striking is the fact that the β_3 subtype is resistant to the agonist-induced short-term desensitization initiated for the β_2 receptor by phosphorylation target sequences absent in β_3.

Diversity in the three βAR subtypes clearly suggests different functional roles in the various tissues in which these receptors are expressed together or alone.

REFERENCES

1. EMORINE, L. J., S. MARULLO, M. M. BRIEND-SUTREN, G. PATEY, K. TATE, C. DELAVIER-KLUTCHKO & A. D. STROSBERG. 1989. Molecular characterization of a new human β-adrenergic receptor involved in catecholamine control of metabolism. Science **245:** 1118–1121.
2. NAHMIAS, C., N. BLIN, J-M. ELALOUF, M. G. MATTEI, A. D. STROSBERG & L. J. EMORINE. 1991. Molecular characterization of the mouse β_3-adrenergic receptor: Relationship with the atypical receptor of adipocytes. EMBO J. **10:** 3721–3727.
3. GRANNEMAN, J. G., K. N. LAHNERS & A. CHAUDHRY. 1991. Molecular cloning and expression of the rat β_3-adrenergic receptor. Mol. Pharmacol. **40:** 895–899.
4. MUZZIN, P., J. P. REVELLI, F. KUHNE, J. D. GOCAYNE, W. R. MCCONBIE, J. G. VENTER,

J. P. GIACOBINO & M. C. FRASER. 1991. An adipose tissue-specific β-adrenergic receptor. Molecular cloning and down-regulation in obesity. J. Biol. Chem. **266:** 24053–24058.

5. BLIN, N., C. NAHMIAS, M-F. DRUMARE & A. D. STROSBERG. 1994. Mediation of most atypical effects by species homologues of the β_3-adrenoceptor. Br. J. Pharmacol. **112:** 911–919.
6. EMORINE, L. J., N. BLIN & A. D. STROSBERG. 1994. The human β3-adrenoceptor: The search for a physiological function. Trends Pharmacol. Sci. **15:** 3–7.
7. BLIN, N., L. CAMOIN, B. MAIGRET & A. D. STROSBERG. 1993. Structural and conformational features determining selective signal transduction in the β3-adrenergic receptor. Mol. Pharmacol. **44:** 1094–1104.
8. STROSBERG, A. D. 1993. Structure, function and regulation of adrenergic receptors. Protein Sci. **12:** 1198–1209.
9. VAN SPRONSEN, A., C. NAHMIAS, S. KRIEF, M-M. BRIEND-SUTREN, A. D. STROSBERG & L. J. EMORINE. 1993. The human and mouse β_3-adrenergic receptor genes: Promoter and intron/exon structure. Eur. J. Biochem. **213:** 1117–1124.
10. LELIAS, J. M., M. KAGHAD, M. RODRIGUEZ, P. CHALON, J. BONNIN, B. DUPRÉ, M. DELPECH, M. BENSAID, G. LEFUR, P. FERRARA & D. CAPUT. 1993. Molecular cloning of a human β_3-adrenergic receptor cDNA. FEBS Lett. **324:** 127–130.
11. GRANNEMAN, J. G., K. N. LAHNERS & A. CHANDLEY. 1993. Characterization of the human β_3-adrenergic receptor gene. Mol. Pharmacol. **44:** 264–270.
12. GRANNEMAN, J. G., LAHNERS & D. D. RAO. 1992. Rodent and human β_3-adrenergic receptor genes contain an intron within the protein coding block. Mol. Pharmacol. **42:** 964–970.
13. KRIEF, S., F. LÖNNQVIST, J. RAIMBAULT, B. BAUDE, A. VAN SPRONSEN, P. ARNER, A. D. STROSBERG, D. RICQUIER & L. J. EMORINE. 1993. Tissue distribution of β_3-adrenergic receptor mRNA in man. J. Clin. Invest. **91:** 344–349.
14. BENOVIC, J. L., M. BOUVIER, M. G. CARON & R. J. LEFKOWITZ. 1988. Regulation of the adenyl cyclase-coupled β-adrenergic receptors. Annu. Rev. Cell Biol. **4:** 405–408.
15. BOUVIER, M., P. HAUSSDORF, A. DEBLASI, B. F. O'DOWD, B. K. KOBILKA, M. G. CARON & R. J. LEFKOWITZ. 1988. Removal of phosphorylation sites from the β_2-adrenergic receptor delays onset of agonist-promoted desensitization. Nature **333:** 370–373.
16. BENOVIC, J. L., A. DEBLASI, W. C. STONE, M. G. CARON & R. J. LEFKOWITZ. 1989. β-adrenergic receptor kinase: Primary structure delineates a multigene family. Science **246:** 235–240.
17. HAUSDORFF, W. P., M. HNATOWICH, B. F. O'DOWD, M. G. CARON & R. J. LEFKOWITZ. 1990. A mutation of the β_2-adrenergic receptor impairs agonist activation of adenylate cyclase without affecting high affinity agonist-binding. J. Biol. Chem. **265:** 1388–1393.
18. HADCOCK, J. R. & C. C. MALBON. 1988. Down-regulation of β-adrenergic receptors: Agonist-induced reduction in receptor mRNA levels. Proc. Natl. Acad. Sci. USA **85:** 5021–5025.
19. VALIQUETTE, M., H. BONIN, M. HNATOWICH, M. G. CARON, R. J. LEFKOWITZ & M. BOUVIER. 1990. Involvement of tyrosine residues located in the carboxyl tail of the human β_2-adrenergic receptor in its agonist-induced down-regulation. Proc. Natl. Acad. Sci. USA **87:** 5089–5093.
20. ZHOU, X. M. & P. H. FISHMAN. 1991. Desensitization of the human β_1-adrenergic receptor: Involvement of the cyclic cAMP-dependent but not receptor-specific protein kinase. J. Biol. Chem. **266:** 7462–7468.
21. SUZUKI, T., C. T. N'GUYEN, F. NANTEL, H. BONIN, M. VALIQUETTE, T. FRIELLE & M. BOUVIER. 1992. Distinct regulation of β_1- and β_2-adrenergic receptors in Chinese hamster fibroblasts. Mol. Pharmacol. **41:** 542–548.
22. NANTEL, F., H. BONIN, L. J. EMORINE, V. ZILBERFARB, A. D. STROSBERG, M. BOUVIER & S. MARULLO. 1993. The human β_3-adrenergic receptor is resistant to short-term agonist-promoted desensitization. Mol. Pharmacol. **43:** 548–555.
23. FÈVE, B., L. J. EMORINE, M.-M. BRIEND-SUTREN, F. LASNIER, A. D. STROSBERG & J. PAIRAULT. 1990. Differential regulation of β_1- and β_2-adrenergic receptor protein and

mRNA levels by glucocorticoids during 3T3-F442A adipose differentiation. J. Biol. Chem. **265:** 16343–16349.

24. FÈVE, B., L. J. EMORINE, F. LASNIER, N. BLIN, B. BAUDE, C. NAHMIAS, A. D. STROSBERG & J. PAIRAULT. 1991. Atypical β-adrenergic receptor in 3T3-F442A adipocytes: Pharmacological and molecular relationship with the human β3-adrenergic receptor. J. Biol. Chem. **266:** 20329–20336.
25. THOMAS, R. F., B. D. HOLT, D. A. SCHWINN & S. B. LIGGETT. 1992. Long-term agonist exposure induces up-regulation of β_3AR exposure via multiple cAMP response elements. Proc. Natl. Acad. Sci. USA **89:** 4490–4494.
26. FÈVE, B., B. BAUDE, S. KRIEF, A. D. STROSBERG, J. PAIRAULT & L. J. EMORINE. 1992. Dexamethasone down-regulates β3-adrenergic receptors in 3T3-F442A adipocytes. J. Biol. Chem. **267:** 15909–15915.
27. KRIEF, S., B. FÈVE, B. BAUDE, V. ZILBERFARB, A. D. STROSBERG, J. PAIRAULT & L. J. EMORINE. 1994. Transcriptional modulation by n-butyric acid of β_1-, β_2, and β_3-adrenergic receptor balance in 3T3-F442A adipocytes. J. Biol. Chem. **269:** 6664–6670.
28. FÈVE, B., K. ELHADRI, A. QUIGNARD-BOULANGÉ & J. PAIRAULT. 1994. Transcriptional down-regulation by insulin of the β3-adrenergic receptor expression in 3T3-F442A adipocytes: A novel mechanism for repressing the cAMP signalling pathway. Proc. Natl. Acad. Sci. USA **91:** 5677–5681.